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SYSTEMATIC INVESTIGATIONS OF BIOMIMETIC CATALYSTS IN THE SYNTHESIS OF REACTIVE METAL OXIDE NANOPARTICLE NETWORKS

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U.S. Army Natick Soldier Research, Development and Engineering Center
Natick, Massachusetts 01760-5020

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14. ABSTRACT

This report discusses research efforts at the Natick Soldier Research Development and Engineering Center (NSRDEC), from 2005 to September 2007, (1) to utilize biomimetic agents to precipitate the metal oxides titania and alumina, (2) to entrap biological agents within the matrixes of these oxides, and (3) to control the morphology of the biomimetically-precipitated titania through biomimetic agent composition. These metal oxides are of interest as potential decontaminating substrates for the hydrolysis and oxidation of chemical agents. The potential to form these compounds in the presence of reactive enzymes and biocidal peptides trapping these agents within the templating matrix provided the motivation for this research, which drew from numerous previous studies that identified some biomimetic approaches for precipitating and templating silica structures found in nature. The results of this study clearly show that silica-precipitating biomimetic agents will precipitate metal oxides beyond silica, show that it is possible to entrap enzymes within the titania matrix and retain activity, and suggest a direction for controlling the morphology of titania precipitation, opening new areas of research and application. This report also suggests several areas for further research.

15. SUBJECT TERMS

T1O2	ENZYMES	NUCLEATION	TITANIUM DIOXIDE
SILICA	BIOCIDES	BIOMIMETICS	ANTIMICROBIAL AGENTS
SILICON	DIOXIDES	METAL OXIDES	
TITANIA	CATALYSTS	SILICONE DIOXIDE	
ALUMINA	OXIDATION	CHEMICAL AGENTS	
PEPTIDES	HYDROLYSIS	BIOCIDAL PEPTIDES	

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

PREFACE

This report documents work that investigated biomimetic approaches to precipitate metal oxides such as titania and alumina. These metal oxides are of interest as potential decontaminating substrates for the hydrolysis and oxidation of chemical agents. The potential to grow these compounds in the presence of agent-reactive enzymes and biocidal peptides within the templating matrix provided the motivation for this research, which was conducted by the the Natick Soldier Research Development and Engineering Center (NSRDEC), between October 2005 and September 2007. This project (No. AA06SPO0004-009) was funded by the Defense Threat Reduction Agency and was administered by the Army Research Office (ARO).

ACKNOWLEDGEMENTS

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SYSTEMATIC INVESTIGATIONS OF BIOMIMETIC CATALYSTS IN THE SYNTHESIS OF REACTIVE METAL OXIDE NANOPARTICLE NETWORKS

1. INTRODUCTION

This report discusses research efforts performed at the Natick Soldier Research Development and Engineering Center (NSRDEC), between October 2005 and September 2007, to precipitate metal oxides using biomimetic agents. Funding was provided by the Defense Threat Reduction Agency (DTRA) in support of their efforts to develop a self-decontaminating uniform.

The research initially had two main objectives: (1) to demonstrate the ability of biomimetic agents to precipitate inorganic oxides other than naturally formed silica and (2) to demonstrate the ability to co-precipitate reactive enzymes with biomimetically-precipitated inorganic oxides. As the project progressed, a third objective was included: (3) to control the morphology of the biomimetically-precipitated titania through biomimetic agent composition.

Chapters 2, 3, and 4 discuss the efforts related to each of the three objectives, respectively. Each chapter describes the methods used to accomplish the objective, presents the results, and discusses those results. Chapter 5 summarizes the findings of this study and recommends four areas for future research.

1.1 Background

Nature has the ability to form intricate structures of silica in an aqueous environment at neutral pH and with low temperature. This is in contrast to current industrial methods, which provide limited control over morphology and involve harsh acids or bases, with high temperatures and pressures. Silica-forming organisms have been studied by various groups in order to gain a better understanding of how nature controls silica formation [1-3]. Two organisms, the diatom and the sea sponge *Tethya aurentia*, have been of particular interest. Both organisms use proteins for precipitation and control, but the mechanisms utilized by each organism appear to be different .

Diatoms precipitate silica using a combination of the peptide silaffin and the polyamine polypropyleneimine. The peptide and polyamine act as polycationic agents forming Lewis acid/base complexes with silicic acid. The silicic acid is rapidly hydrolyzed within the complexes followed by condensing to the silica [4]. The diatom controls morphology through varying the composition of the silaffin sequence and varying the chain length of the polyamine [1, 2]. In laboratory experiments, both the silaffin peptide and the polyamines have been shown to effect the precipitation of silica [5]. Of equal importance, when an enzyme was present during the silica formation, the enzyme was entrapped within the silica matrix and retained its activity [6-8].

The sea sponge forms silica spicules using Silicatein, an enzymatic protein from the Cathepsin L subfamily [3]. The Cathepsin L subfamily is a group of lysosomal proteolytic enzymes which normally act as proteases. Unlike the other enzymes in the family, Silicatein acts upon a small molecule, silicic acid, rather than other proteins. Condensation of silica spontaneously commences once hydrolysis of silicic acid molecules has begun. Due to the enzyme's binding pocket, which only binds one silicic acid molecule at a time, the concentration of hydrolyzed silicic acid molecules is low, resulting in a slower silica formation rate than seen with the silaffin peptide.

Using the mechanistic knowledge obtained from the diatom and sponge studies, several alternative agents have been identified as potential biomimetic agents for silica precipitation. Block co-polymers, small biomolecules, and polyamines have all demonstrated the ability to form silica in vitro using a similar mechanism to that seen with the native agents, but the control is more variable [9-12]. More recent studies have demonstrated that some silica-precipitating agents have the ability to form oxides from different metals, including gallium, titanium, and zinc [13-15]. The general mechanism for the precipitation of these oxides appears to be similar, but the chemical properties of the metals play a role in the precipitation.

2. DEMONSTRATING FORMATION OF METAL OXIDES USING BIOMIMETIC TEMPLATES

This chapter describes the work related to the first objective of this study. These efforts involved investigation of whether silica-precipitating agents would precipitate titania. Biomimetic agents were identified as potential nucleating agents for titanium dioxide and aluminum oxide. Numerous polymeric templates (Table 1), such as polyallyamine (PAA), were investigated, along with amine-functionalized surfaces such as DEAE Sephadex™.

Table 1 Polymers tested.

Polymer	Avg. Molecular Weight	Manufacturer
PAA-H-10C (PAA)	59,900 Da	Nitto Boseki, LTD
Erkol@ M12 (vinyl alcohol/vinyl amine)	80-140 kDa	Erkol, S.A
Basocoll@ (vinyl amine and N-vinylformamide)	Two fractions	BASF
Catiofast@ PR8106 (polyvinylamine-HCl)	N/A	BASF
Poly-L-lysine	4,000-10,000	Sigma
PAA	70,000	Sigma-Aldrich

2.1. Methods for Precipitating Oxides

The reaction mixture for each polymer tested contained 800 µL of 25 mM tris HCl at pH 7.2 with 100 µL of the polymeric template (10% wt/vol) mixed with 100 µL of 50% wt/vol titanium (IV) bis-(ammonium lactate)-dihydroxide (TBALD). The reaction mixtures were incubated at room temperature for 24 h with mild agitation. Any precipitates were collected via centrifugation at 10,000 rpm for 2 min and washed three times in milli-Q water. Samples were air dried. Analysis of the precipitations was performed using scanning electron microscopy (SEM)/ energy dispersive spectroscopy (EDS) (a Zeiss Evo 60 EP-SEM, equipped with an EDAX Genesis EDS X-ray detector) and X-ray diffraction (a Bruker-AXS D8 Advance X-ray diffractometer).

DEAE Sephadex™, Sephadex™ LH60 (an anionic functionalized resin) and Sephadex™ G-100 (unfunctionalized) were used as the functionalized surface. The reaction conditions were similar to those listed above except the Sephadex™ resins were swelled in the 900 µL of tris buffer overnight before use. All other steps were the same including incubation and washing.

The templates listed in Table 1 were investigated for their ability to precipitate alumina. The reaction conditions were similar to those utilized for the titania studies. Instead of TBALD, 100 µL of 1 M aluminum nitrate was used as the precursor. Also before the precipitates were collected via centrifugation, some of the reactions were first concentrated using a Millipore centricon unit. Any precipitates were collected by centrifugation and washed with water. The washed mixtures were either dried using a SpeedVac (Savant Instruments, Hicksville, NY) or air dried. Analysis of the precipitates was performed using SEM/EDS (a Zeiss Evo 60 EP-SEM, equipped with an EDAX Genesis EDS X-ray detector) and X-ray diffraction (a Bruker-AXS D8 ADVANCE X-ray diffractometer).

2.2. Results

Titanium-containing precipitates formed with all of the polymers tested and from some of the functionalized surface. SEM images (Figure 1) show that with the exception of the precipitates formed with the native silaffin and Erkol, the precipitate universally formed as fused sheets. The native silaffin and the Erkol formed precipitates that not only contained sheets, but some particulates as well. Both materials had regions that were platelike and regions that were particulate. Changing pH or temperature did not demonstrate a significant effect on the morphology of the precipitates. EDS analysis and X-ray diffraction confirmed that the precipitates were primarily an amorphous form of anatase with some nanocrystallinity.

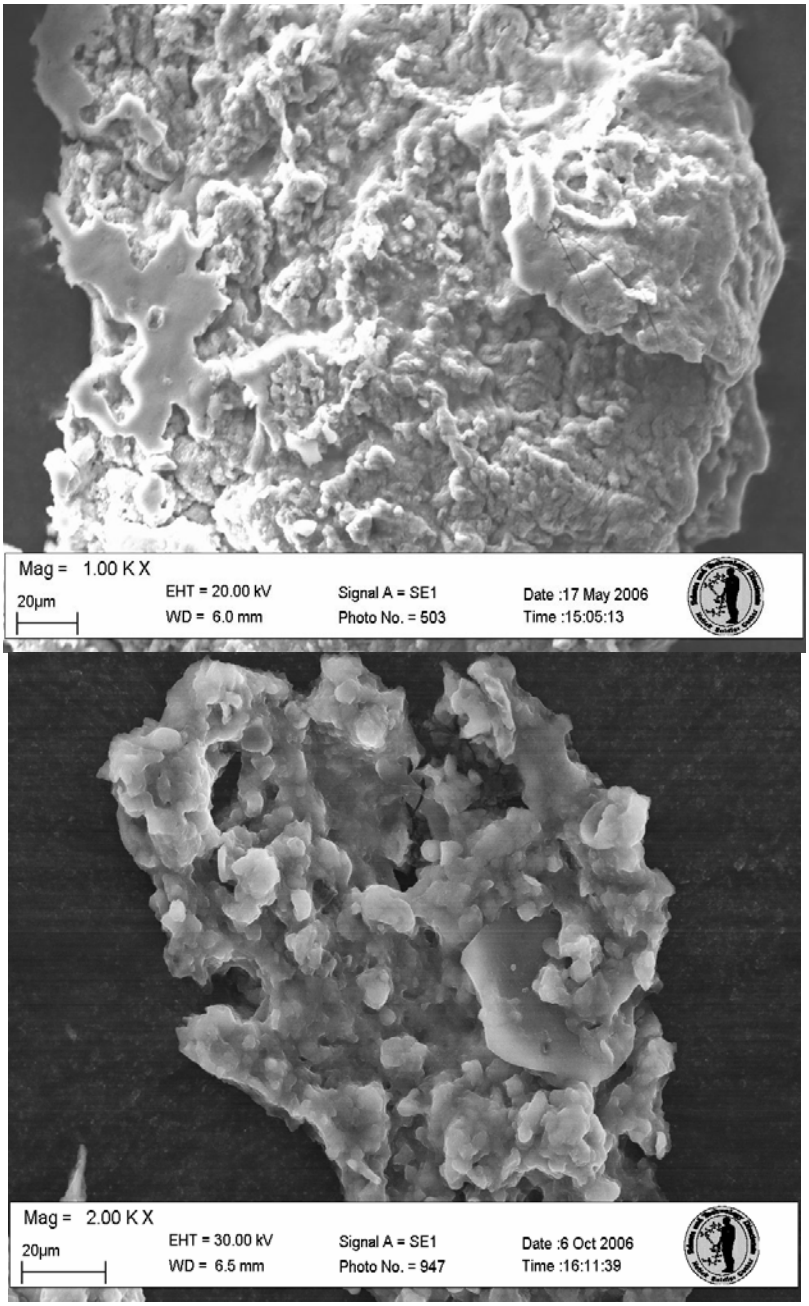

Figure 1. SEM images of Erkol (top) and silaffin (bottom).

Both the DEAE Sephadex™ and the G100 Sephadex™ materials precipitated titania, but the LH 60 Spehadex™ did not. SEM images show that the precipitate for the G100 and the DEAE Sephadex™ materials formed as a coating around the resin. The images in Figures 2a and 2b are the precipitate for the DEAE without and with exposure, respectively, of the resins to TBALD at pH 7.2. Some cracking of the coating was seen in the SEM images, as shown for the DEAE in Figure 2b. EDS analysis (shown for the DEAE in Figure 2, bottom) confirmed the presence of the titanium and oxygen as the primary components of the coatings.

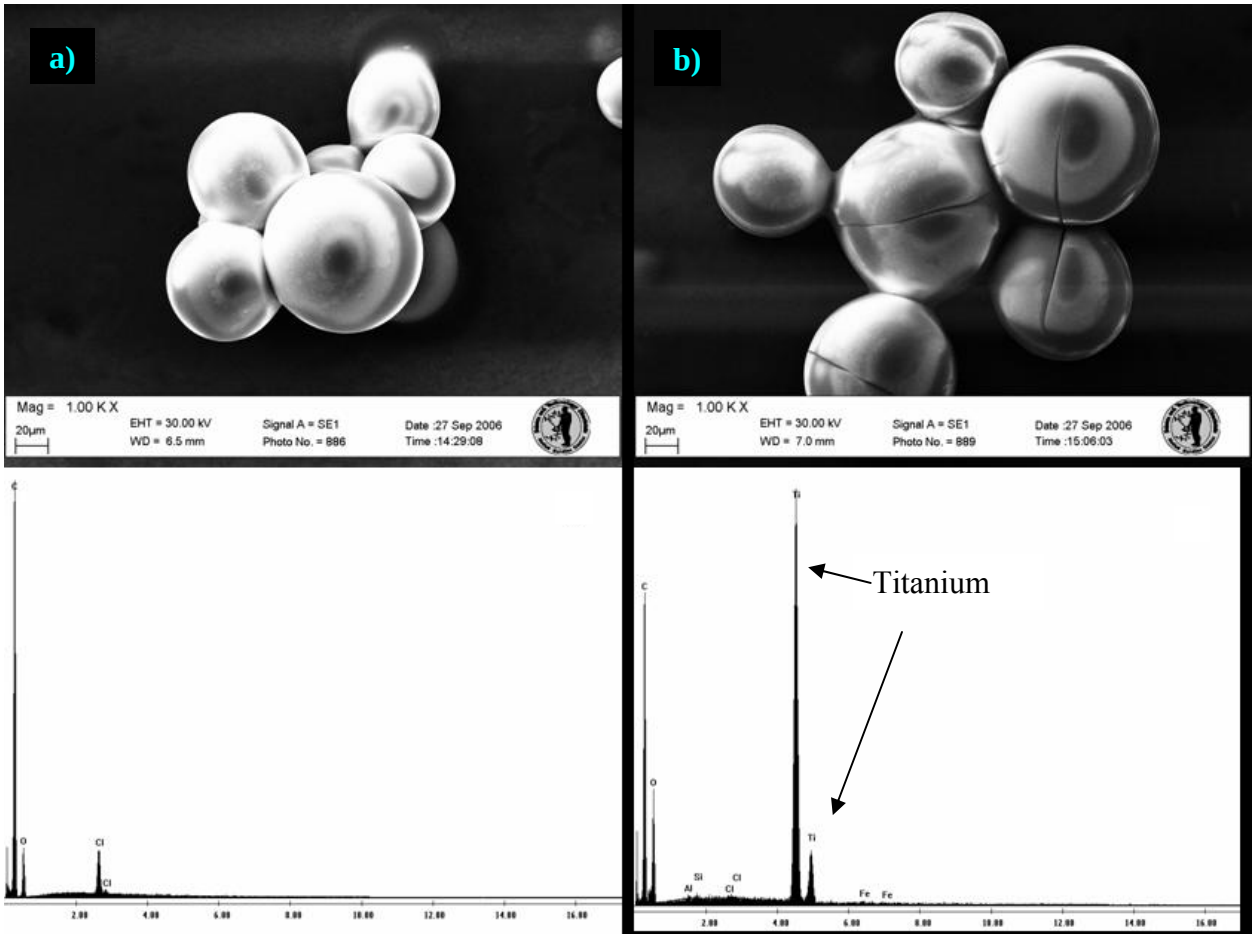

Figure 2. SEM images of DEAE Sephadex™ (top) and EDS results (bottom):
a) Without exposure to TBALD at pH 7.2; b) With exposure to TBALD at pH 7.2.

When tested using PAA, aluminum nitrate quickly formed a white precipitate that dissolved back into the solution with time. After the literature was reviewed, a drying step was added, using a cryogenic SpeedVac (Savant Instruments, Hicksville, NY), to remove the water and reform the precipitate. The X-ray analysis of the resulting precipitate showed a composition consisting of multiple materials, of which some peaks lined up to those associated with alumina, aluminum hydroxide, and aluminum nitrate. Figure 3 shows the SEM image and the EDS and X-ray diffraction results for the precipitation of aluminum hydroxide. The numerous peaks suggest multiple crystal types.

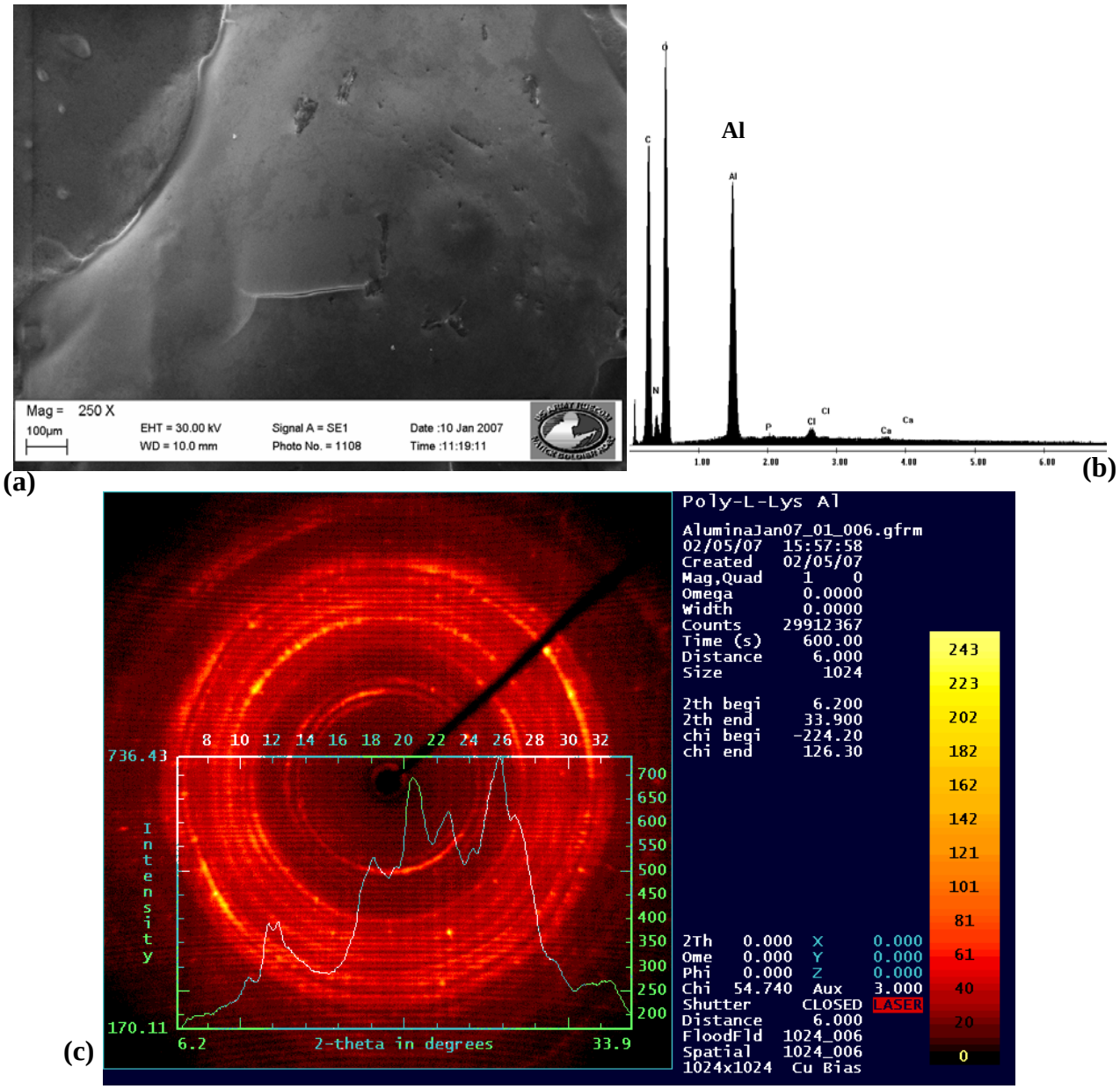

Figure 3 Precipitate obtained from biomimetic precipitation of aluminum hydroxide: (a) SEM image; (b) EDS results; (c) X-ray diffraction results.

2.3 Discussion

As with silica, the amine groups acted as Lewis bases, binding to the titanium and hydrolyzing the TBALD complex. Unlike silicic acid, the hydrolyzed TBALD is unstable and quickly stabilizes by binding to another titanium complex. The resulting precipitate is more sheet-like, composed of fused plates instead of particulates as had been seen with the silica. X-ray analysis showed the precipitate to be an amorphous form of anatase with some nanocrystallinity. The literature suggests that the reaction could be slowed with the addition of glycerol. This was tried at various ratios of glycerol, with no change to the morphology of the titania (results not shown). The results from the DEAE Sephadex ™ experiments seem to benefit from the rapid precipitation.

DEAE Sephadex ™ is an amine-functionalized resin normally used for separating proteins by charge. Sephadex™ G-100 (unfunctionalized) and Sephadex™ LH60 (an anionic functionalized lipophilic resin) have no amines, but have hydroxyls on their surface instead. It was expected that the amines on the surface of the DEAE Sephadex™ would precipitate titania, which was seen by SEM and confirmed through EDS. However, the precipitation of titania by the Sephadex™ G100, though a lesser amount than by the DEAE, was a surprise. Hydroxyl groups are weak Lewis bases and may be capable of precipitating titania. For the Sephadex ™ G100 this seems to be true. Sephadex™ LH60 also has hydroxyl groups, but failed to precipitate any titania. The Sephadex™ LH60 is a functionalized material that is lipophilic, making the hydroxyl more sterically-hindered than those seen on the Sephadex™ G100. This would make the LH60 hydroxyl less accessible for binding the TBALD and unable to precipitate titania.

There are no published papers on precipitating alumina using biomimetic agents, but a study was reported on precipitating gallium oxide using biomimetic agents[13]. In that study, water-soluble gallium nitrate was hydrolyzed to the water-insoluble gallium hydroxide using a silicatein mimic. The gallium hydroxide was further condensed to the insoluble gallium oxide as water was removed. Aluminum falls in the same group of the periodic table as gallium and therefore has similar chemical and physical properties.[16] Using the gallium study as a starting point, aluminum nitrate was solubilized into tris, and then PAA (a silaffin mimic) was added to the reaction. A precipitate was immediately formed, but slowly dissolved back into solution over the course of the 24-h reaction. Drying/condensing the reaction mixture caused the precipitate to re-form, allowing the precipitate to be collected by centrifugation.

Similar to the gallium, the water-soluble aluminum nitrate would initially be hydrolyzed by the PAA to form the water-insoluble aluminum hydroxide ($Al(OH)_3$). This was the precipitate seen at the start of the reaction. However, unlike gallium hydroxide, aluminum hydroxide would further hydrolyze to form the water-soluble tetrahydroxide form $Al(OH)_4$, which is why the precipitate redissolved over time. Removing water from the reaction should push the tetrahydroxide back to the hydroxide form and then to the oxide. Using a Sorval SpeedVac, water was evaporated from the reaction mixture. As the water evaporated, a precipitate was formed consistent with the shift from the tetrahydroxide to the hydroxide. The precipitate collected by centrifugation was further dried in an attempt to move the hydroxide to alumina. X-ray diffraction of the dried precipitate showed the precipitate was a mix of several different materials, including but not limited to alumina, aluminum hydroxide, and the precursor aluminum nitrate (Figure 3). Similar results were seen when other polyamines were tried, such as polylysine. The presence of the aluminum nitrate precursor suggests there was an excess of the precursor, which could not be hydrolyzed by the PAA. As the precipitate formed, some of the unreacted precursor became entrapped within the precipitate and was not washed away during the centrifugation step. The presence of aluminum hydroxide in the final precipitate indicates the Sorval SpeedVac was not sufficient for drying the material to push all the hydroxide to alumina. The presence of the unidentified peaks suggests there was more chemistry occurring beyond the hydroxide-oxide reaction, most likely with the polymer or the buffer. Due to time constraints, biomimetically-precipitating alumina was not pursued further, but there were several conditional changes that could possibly improve the precipitate purity, including but not limited to reagent concentration, buffer, and longer drying times.

3. ENTRAPMENT OF ENZYMES WITH IN METAL OXIDES MATRICIES

This chapter describes the work related to the second objective of this study: to demonstrate the ability to co-precipitate a reactive enzyme with biomimetically precipitated inorganic oxides. Formation of titania under mild aqueous conditions opens the possibility for entrapping biomolecules and chemically sensitive compounds within the titania crystalline matrix by co precipitating the agent with the titania. Efforts to entrap the chemical reactive enzyme DFPase into biomimetically-formed titania, using PAA or DEAE Sephadex™, are described.

3.1 Method

The reaction method for incorporating enzymes was similar to those described in Chapter 2. Each reaction was composed of 700 µL of 25 mM tris HCl at pH 7.2 with 100 µL of the PAA (10% wt/vol), 100 µL of 250 mg/mL DFPase, and 100 µL of 50% wt/vol TBALD. When the DEAE Sephadex™ beads were used, 25 mg of dried resin beads were swelled in 800µL of 25 mM tris overnight, and then 100 µL of 250 mg/ml DFPase and 100 µL of 50% wt/vol TBALD were added to the mixture. The reaction was incubated at room temperature for 24 h with mild agitation. Any precipitates were collected via centrifugation at 10,000 rpm for 2 min at room temperature and washed three times in Milli Q water. The beads and precipitate were dried using a Sorval SpeedVac.

DFPase activity was measured using the method described by Hoskins [42]. Test samples were re-suspended in 345 µL of 0.01 M Piperazine-N,N′-bis(2-ethanesulfonic acid)(PIPES), pH 7.2. A fluoride ion probe was placed in the sample and allowed to equilibrate. Once equilibrated, a 150 µL aliquot of a 0.01 M DFP solution in MQ water was added to the mixture. Fluoride ion evolution measurements were recorded at 1-min intervals. A baseline was established prior to the assay by adding a 150 µL aliquot of DFP to 345 µL of buffer and allowing the mixture to equilibrate. A 5 µL aliquot of DFPase (250 mg/mL) was added to the mixture, and measurements were taken until the fluoride concentration reached 250×10^{-5} M.

To determine the thermal stability of the entrapped enzyme, three sets of beads were prepared with titania-entrapped DFPase. For each set, 50% of the titania-DFPase-coated DEAE Sephadex™ beads were left with residual water (wet sample), while the other half of the beads were freeze dried prior to the experiment (dry sample). One set was incubated at 30°C (room temperature), 50°C, or 90°C for 24 h. After incubation, the heated beads were tested for activity against DFP as described above.

The residual 2-chloroethyl ethylsulfide (CEES) on the samples was analyzed as an indicator of CEES decomposition rates on these surfaces. Vapor exposure tests for CEES using biomimetically-precipitated titania samples were conducted by placing 250 mg of titania product into a closed vial with a disk of filter paper contaminated with 5 µL of CEES. The sample was allowed to absorb the CEES vapors and react with the CEES for 24 h. Weight gains for each sample were determined in a separate adsorption experiment, allowing 10 mg of sample to adsorb 5 mg of CEES from a liquid-contaminated filter paper source over a 24 h period. CEES and any reaction products were removed from the titania through thermal de-absorption and analyzed using a gas chromatograph (GC).

3.2 Results

The results show that titania-entrapped DFPase removed the fluoride from DFP (Figure 4). The control showed a low level of fluoride ion production from self-hydrolysis of DFP in buffer until neat DFPase enzyme was added at the 9-min mark; rapid fluoride ion flux was seen at that point. The four titania samples each showed a similar low level of fluoride ion production without DFPase. DFPase entrapped in both the PAA and DEAE Sephadex™ showed activity against DFP when added at the 5-min equilibration mark, though the DEAE Sephadex™-entrapped DFPase exhibited greater activity than the DFPase entrapped using the PAA.

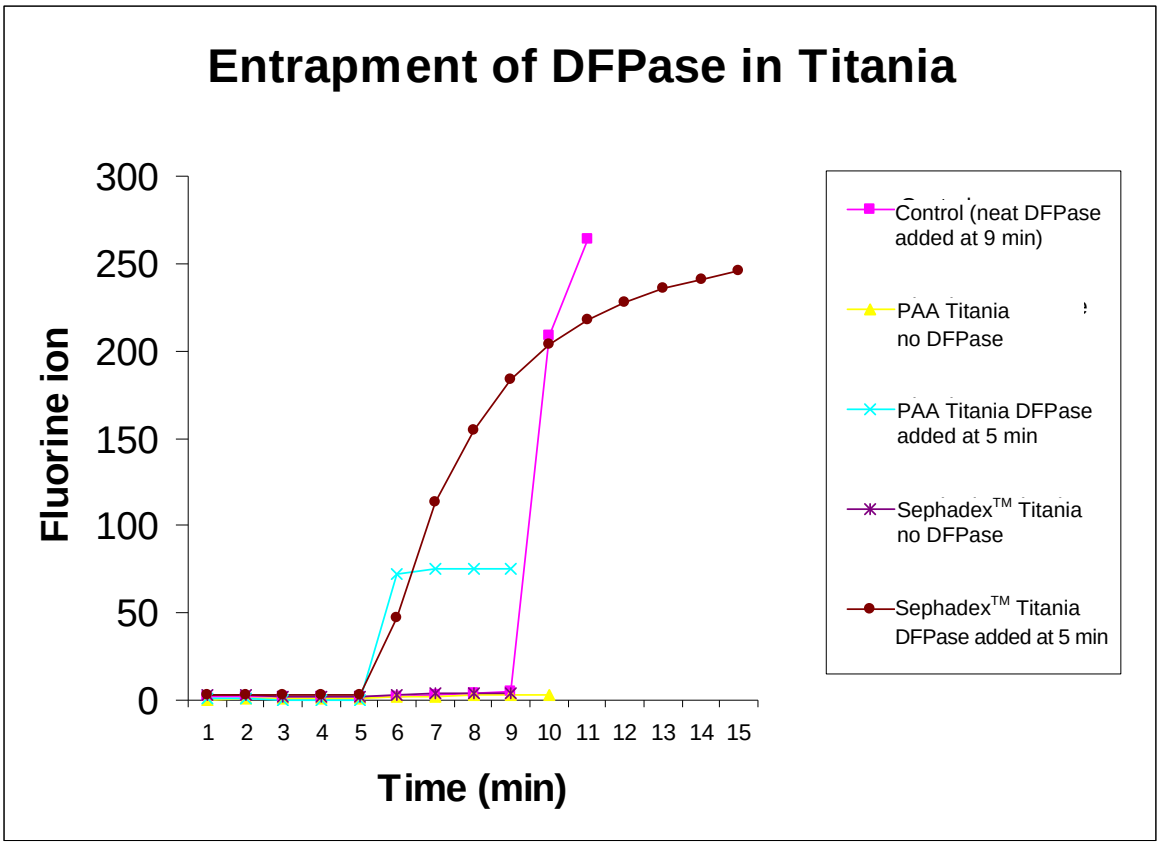

Figure 4. Activity results for DFPase entrapped in biomimetically precipitated titania.

Studies of the thermo-stability of the entrapped DFPase revealed a loss of activity for DFPase after heating. This was most evident when the material was left wet. It lost almost all of its activity over the 24-h period. Dry heated material retained a greater percentage of its activity than the wet samples. Results of DFP hydrolysis testing for the DEAE Sephadex™ (Figure 5) suggest there was good heat stability of the entrapped enzyme in the dry samples, but loss of enzyme activity when the material was wet and heated to 90°C Results of DFP hydrolysis testing of the PAA (Figure 6) show that the stability of the precipitated material decreased as the heat rose.

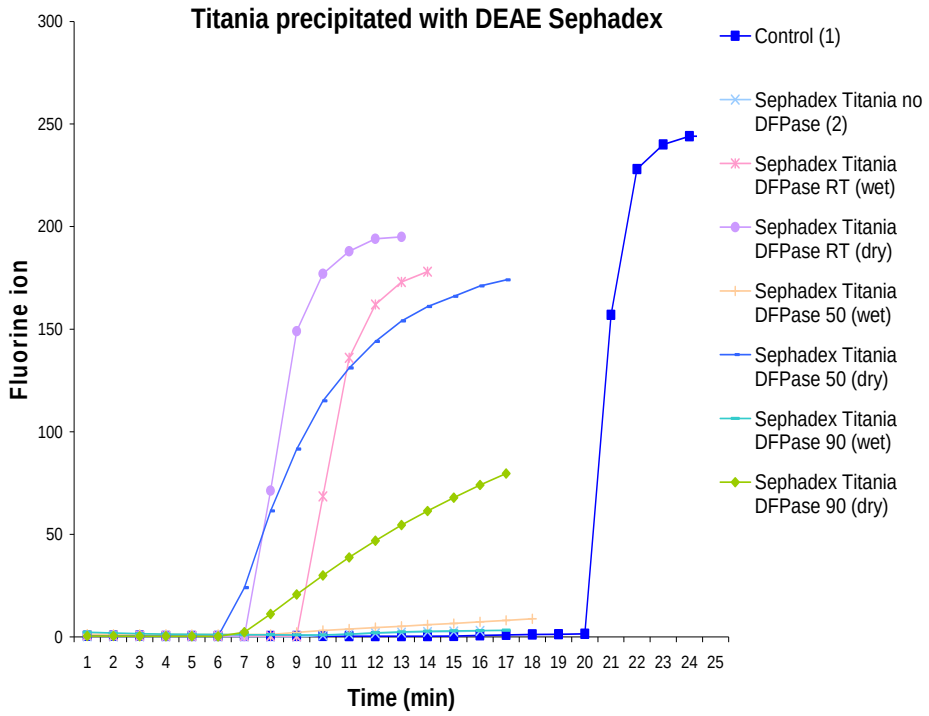

Figure 5. DFP hydrolysis testing with DEAE-Sephadex™-precipitated nanocrystals after heating to 50 °C and 90°C.

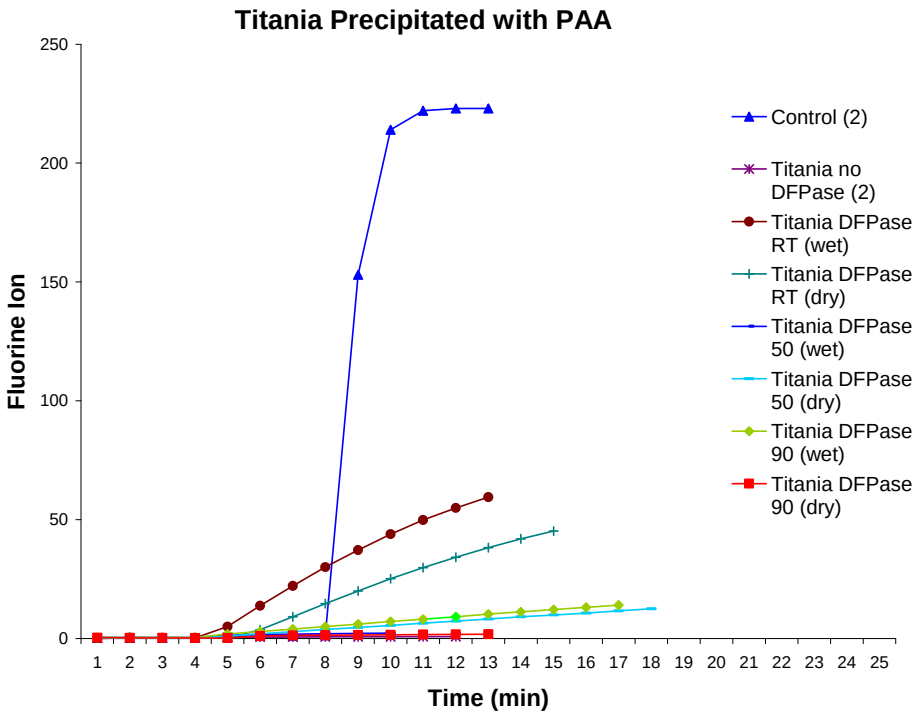

Figure 6. DFP hydrolysis testing with PAA-precipitated nanocrystals after heating.

Crystalline titania had been shown to be capable of breaking down CEES when dry. Biomimetically-precipitated titania with its nanocrystallinity had not been tested against CEES, so it was unknown if there would be any degradation by the biomimetically-precipitated titania. A CEES test was conducted using the dry biomimetically-precipitated titania, both bulk and coated

onto Sephadex™. For each material two sets were prepared, one with DFPase and one without. NanoActive titania was run as a positive control. The results show that biomimetically-precipitated titania with DFPase did start to breakdown the CEES, though not as completely as the NanoActive titania. Each sample adsorbed from 19 - 28% by weight of CEES vapor. The thermal desorption GC results (Table 2, Figure 7) show the influence of the templating matrix on the performance of the TiO_2 to dehalogenate the 2-CEES to 2-(ethylthio)ethanol and towards forming the dimer, 1,2-bis(ethylthio)ethane. Both the bulk biomimetic titania and the titania-coated DEAE Sephadex™ were active against the CEES as long as the DFPase was present. The activity was minimal for the biomimetically-precipitated titania without the enzyme. Only the commercial sample of nanoTiO_2 produced significant amounts of the diethylsulfide, which results from binding surface hydroxyls on the surface of the titania followed by rearrangement of the 2-CEES.

Table 2. GC peak heights of adsorbed/reacted CEES and CEES reaction products thermally desorbed from contaminated templated TiO_2.

TiO2 Sample Template Name	CEES Adsorb. (%)	CEES Remaining (GC counts)	Diethyl sulfide (GC counts)	bis(2-ethylthio) ethane (GC counts)	2-(ethylthio) ethanol (GCcounts)
TBALD/PAA	20.3	56,696,369	2,906,831	68,136,032	243,957,529
TBALD/DEAESephadex	19.5	11,204,958	3,728,928	21448928	107,757,534
TBALD/PAA/DFPase	27.5	16,654,851	1,494,618	27538129	377,819,940
TBALD/DEAESephadex/DFPase	23.3	13,384,121	4,740,160	21331904	154,457,868
DEAESephadex/DFPase	28.2	24,852,799	651,414	15,933,738	56,478,794
NanoActive TiO_2	23.2	3,764,443	52,004,872	3117986	36,501,448
Sand	13.4	26,450,730	1,717,928	8,356,543	3,997,083

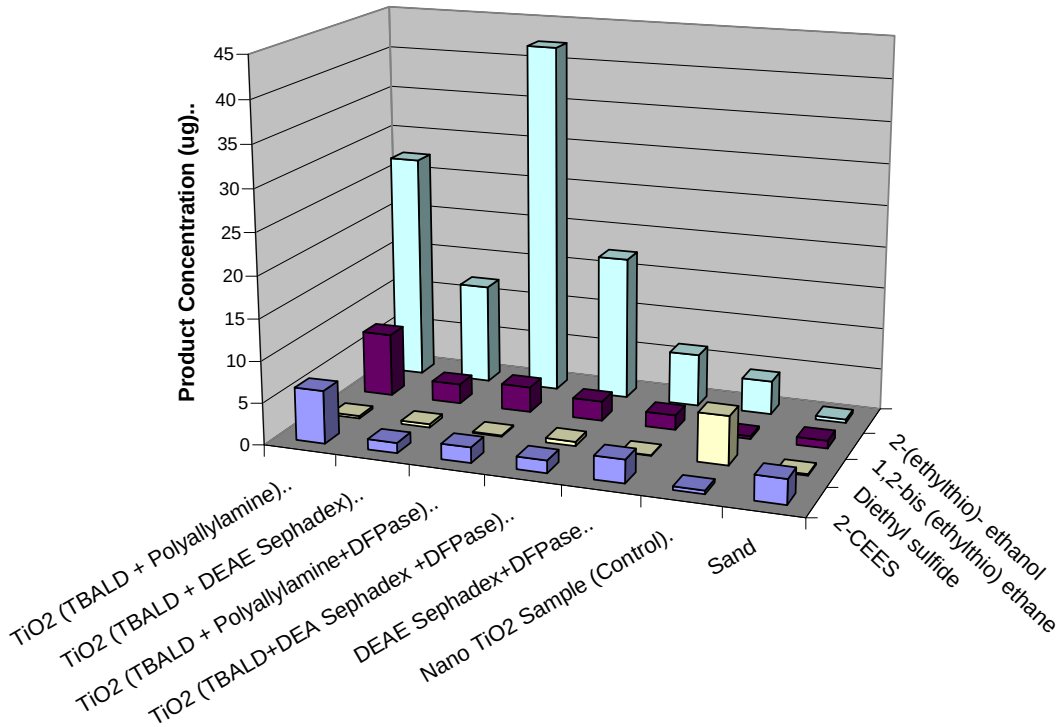

Figure 7. Residual 2-CEES and reaction products after 24-h contamination of half-mustard vapor on templated titania samples compared to commercially obtained nanoTiO_2 control sample (NanoActive TiO_2).

3.3 Discussion

Titania-entrapped DFPase demonstrated the ability to dehalogenate DFP in both the bulk precipitation and the surface of DEAE Sephadex™ beads. The activity for the beads was greater than that seen for the bulk precipitation. Given that the bulk material has a higher percentage of titania per weight, the bulk was expected to exhibit a greater rate of DFPase activity. However, much of the DFPase was trapped within the bulk making the enzyme inaccessible. The DEAE Sephadex™ forms titania on the surface of the beads as a thin coating. Any entrapped DFPase would therefore be on the surface making the enzyme more available for acting upon the DFP, thus allowing for a greater perceived rate than was seen with the bulk material.

DFPase entrapped on the surface of DEAE Sephadex™ beads was tested for heat stability. Much of the enzyme activity was lost when the beads were stored at elevated temperatures. Beads stored wet were especially affected by the heat, losing most of their activity at 90°C. This was an unexpected and disappointing result. There are a couple of possible explanations. The titania itself may have broken down the DFPase. Titania is known as an oxidizer when irradiated with UV light. These samples were not stored in the dark, so there would be some UV light present, which could have degraded the DFPase. This would explain the activity loss seen for the materials. Another possible explanation is that the enzyme may have been affected by the breakdown of the Sephadex™ beads by the titania.

The associated literature for the DEAE Sephadex™ suggests the resin is sensitive to oxidizers. With increased heat, the breakdown of the Sephadex™ beads by the titania would be accelerated, making it more apparent than in room temperature storage. One of the breakdown products from the degraded beads is an acid that DFPase is sensitive to. The greater loss of activity seen in the wet sample would be expected if the Sephadex™ resins are indeed degrading to an acid.

The CEES results were encouraging. The biomimetically-precipitated titania did initiate the breakdown of CEES with the addition of the DFPase, though it did not bring the CEES to the final product of diethylsulfide, as seen with the NanoActive titania. The NanoActive titania control was a crystalline form of titania. The crystal region is the site for CEES degradation. Biomimetically-precipitated titania is primarily amorphous with some nanocrystaline regions. Alone, these nanocrystallines were not sufficient to provide a significant amount of degradation of the CEES. However, with entrapped DFPase the biomimetically-precipitated titania showed an increased presence of 2-(ethlthio)ethanol, suggesting the initiation of CEES degradation. These samples were tested dried, and DFPase would normally not be expected to be active, since it requires water to dehalogenate samples. The activity by the DFPase in these dried samples suggests the titania matrix entrapped not only the DFPase, but some water as well. This would allow the enzyme to be active and to remove the chlorine from the CEES, initiating CEES degradation. However, the results do not provide evidence that the removal of the chlorine by the DFPase moves the CEES farther down the degradation cycle. Beyond dehalogenation, very few degradation products were isolated from the biomimetically-precipitated titania.

4. ANALYSIS OF CONTROLING MORPHOLOGY WITH SPECIFIC PEPTIDES

Controlling morphology was not one of the original project objectives, but given the unexpected morphology seen in the titania from the silaffin and Erkol precipitations, efforts to control that morphology were added to the scope of this project. Though the titania was precipitated primarily as sheets for both the silaffin and Erkol, unlike the polyamines, there were regions that formed particulates. The silaffin peptide is composed of amino acids, which contain amine side chains, along with hydroxyl, guanidine, and carboxyl side chains. Similarly, Erkol is a co-polymer blend of polyvinyl amine and polyvinyl alcohol, which provides both amine and hydroxyl side chains. It was theorized that the addition of these other side chains allowed the silaffin and the Erkol to break up the precipitations, forming particles along with fused sheets. It was further theorized that it would be possible to control the morphology of the titania precipitate by controlling the composition of the nucleating element. This chapter reports and discusses the results from efforts to the control morphology of biomimetically-precipitated titania using peptides with controlled sequences.

4.1. Method

Peptide arrays were synthetically produced by New England Peptide, Gardner MA. Four sets of peptides were synthesized. The first set contained systematic variations of amine and hydroxyl side chains using strings of one to four lysines (amines) separated by similarly varying strings of serines (hydroxyls). The second set contained the same systematic variations as the first string except the serines were replaced with alanine (a methyl side chain) to determine whether the hydroxyls played an active or passive role in morphological control. A third set replaced the amine strings from the first set with arginine, which contains a guanidine side chain (a potentially stronger Lewis base). The last set contained variations on the native silaffin peptide to identify important regions. There was a total of 48 peptides.

Testing of the peptides involved re-suspending the peptides in MQ water at a concentration of 100 mg/mL and then adding 15μL of the peptide suspension to one well of a microtiter plate. To each well, 40 μL of 25 mM tris buffer (pH 7.2) was added and mixed with the peptide. To start the reaction, 15 μL of TBALD (50 % wt/vol) was added to each well. The microtiter plate was covered and allowed to incubate for 24 h at room temperature with mild agitation. Any precipitates were collected via centrifugation at 3000 rpm and washed with water. After air drying, the precipitates were loaded onto SEM studs and visualized using the SEM.

4.2 Results

From previous research on polycationic molecules in aqueous environments, it was expected that all these peptides would be able to form titania.[4, 14, 17, 18] With the exception of some of the truncated silaffin peptides, this assumption proved to be true. SEM analysis found that most of the lysine-containing peptides precipitated titania as sheets. The noticeable exceptions were those containing amine strings of four separated by two or more hydroxyl groups. These peptides formed fused particles (Figure 8). More porous structures were seen when the peptide contained fewer hydroxyls while more discrete particulate structures were seen with hydroxyl strings of four. This morphological control was not duplicated when the hydroxyl side chains were replaced by methyl chains. All of the precipitates with alanine formed as sheets.

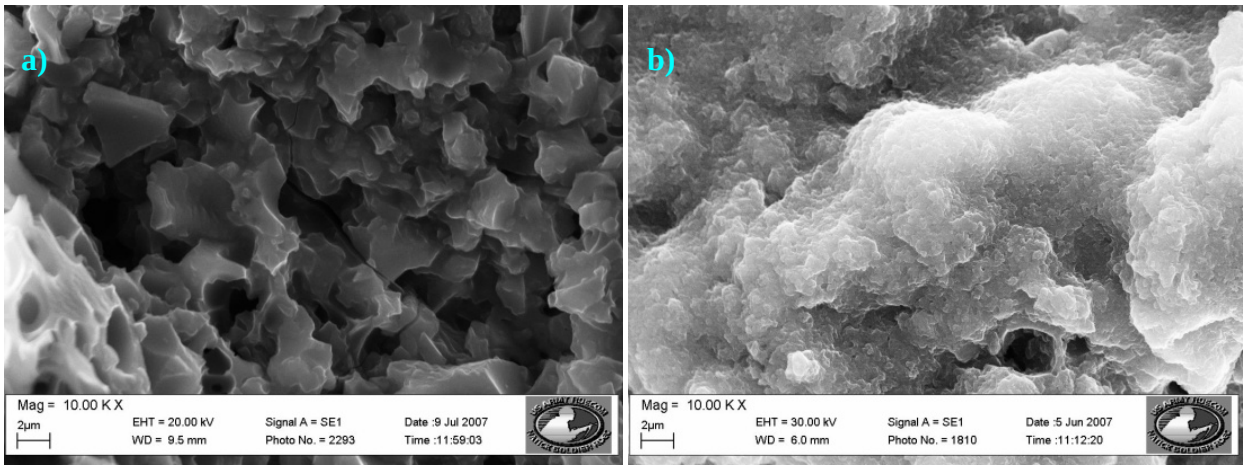

Figure 8. Titania precipitation using synthetic peptides composed of lysine and serine: a) Peptide composed of strings of four lysines and two serines; b) Peptide composed of four lysines and four serines.

The arginine-serine (guanidine-hydroxyl) peptides produced titania precipitates universally as particulates. When one to three arginines were separated by one to two serines, the morphological shapes of the particulates were random. The particulate sizes ranged from 0.5-20 µm for peptides with one to two amines separated by one serine (Figure 9a) and ranges of 2-20 µm for the three arginines separated by two serines (Figure 9e). When one to two arginines were separated by three serines, the precipitate shape became more consistent with an average particle size of 0.5 µm (Figures 9b and 9d), though the peptide containing the strings of two arginines separated by three serines also formed larger balls averaging 3-4 µm (Figure 9d). Under higher magnification, these larger balls were determined to be fusions of the smaller particles. Peptides composed of four arginines separated by only one serine formed consistent particles of 1-2 µm with some degree of fusion (Figure 9c).

The sequence for silaffin is:

S-S-K-K-S-G-S-Y-S-G-S-K-G-S-K-R-R-I-L

Peptides in this set either replaced the hydroxyl or amine/guandine side chains with a methyl side chain or removed amino acids from the N or C terminal of the silaffin. The peptides with the replaced amino acids precipitated titania, but as sheets. This was true whether the serines or the lysine/arginines were replaced. The truncated silaffin peptide set did not universally precipitate titania. Removing up to four amino acids from either end generated peptides that were unable to precipitate titania. Cutting the two serines from the N terminal precipitated titania, but as sheets with no indications of morphological control. Removing the serines and the two lysines from the N terminal created a peptide unable to precipitate titania. Cutting the Isoleucine and the Leucine from the C terminal had no impact on the peptide's ability to precipitate titania nor on its ability to form particles. Once the two arginine residues were removed, the peptide failed to precipitate titania.

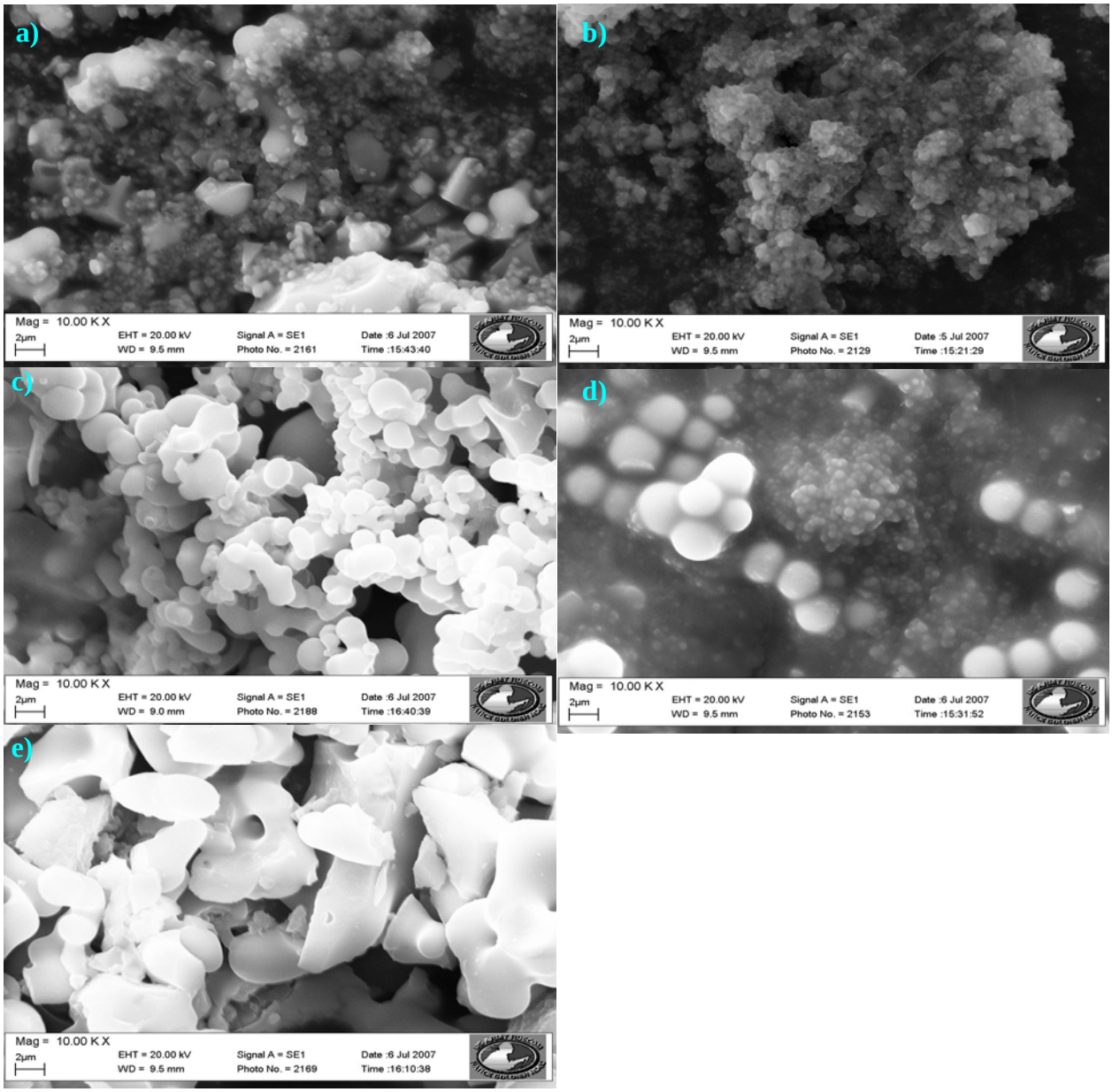

Figure 9. Precipitations using peptides with guanidine groups instead of amines: a) Peptide composed of strings of three arginines separated by a single serine; b) Peptide composed of a single arginine separated by strings of three serines; c) Peptide composed of strings of four arginines separated by a single serine; d) Peptide composed of strings of two arginines separated by strings of three serines; e) Peptide composed of strings of three arginines separated by strings of two serines

4.3 Discussion

Erkol's and silaffin's ability to form particulates along with sections of sheets was significant. It was known that polyamines would precipitate titania as sheets, but the addition of hydroxyl side chains to the Erkol suggested the hydroxyl was the morphological controlling element. The results from the peptide study showed that the hydroxyl side chains were indeed a critical element. Peptides containing strings of four lysines separated by one or more hydroxyls were able to fuse particles, but replacing the hydroxyls with methyl groups provided no control over morphology at all. The role of the hydroxyl was confirmed by the truncated silaffin

peptides. Cutting the serines (with hydroxyl side chains) from the N terminal allowed precipitation, but no particle formed. The precipitates formed as sheets similar to those seen for the polyamines. Though it is not clear how the hydroxyls effected morphological control, part of the mechanism may be linked to the hydroxyl's ability to act as a Lewis base. The slowed hydrolysis may slow part of the precipitation allowing particulate formation, though further testing would be necessary to determine if this is in fact the case.

The role of the guandine side chains is not as clear as the roles of the amine and hydroxyl side chains. Guanidine is a stronger Lewis base than the amine and certainly is capable of precipitating titania. Unlike the amines, the precipitate forms as small particle with or without the hydroxyls. Earlier work using poly-arginine precipitated titania as small particles consistent with these results, but adding the hydroxyl side chains affected the particle size. In general it appears that strings of one to two arginines separated by three serines yielded small particles while strings of four arginines separated by one serine yielded larger ones. How the hydroxyls impact the particle sizes is not clear, but it is reasonable to assume the hydroxyls affect the arginine in a similar fashion as they affect the lysine.

The results from the truncated silaffin peptides confirm the suggestion that the hydroxyls control morphology, but also suggest the number of amines/guandine is important to precipitation. The removal of four amino acids from either terminal produced peptides that no longer had the ability to precipitate titania. This corresponds to the removal of either two amine groups or two guanidine groups from the peptide, leaving only four amine/guanidine groups in the peptide. This is a critical number for titania precipitation. Earlier work using short strings of lysine (two to five residues) showed that strings of five lysines were capable of precipitating titania, but strings of less than five lysines were unable to precipitate the titania. Removing two amines or guanidine reduced the precipitating elements to four, changing the critical number from five to four. This is not a function of peptide size since the truncated silaffin is a 15mer, which is three times larger than the 5mer lysine. It does indicate there is a critical need to have a specific number of strong Lewis bases to initiate precipitation.

5. SUMMARY AND RECOMMENDATIONS OF FUTURE WORK

The results presented clearly show that silica-precipitating biomimetic agents will precipitate metal oxides beyond silica. The results further show that it is possible to entrap enzymes within the titania matrix and retain activity. Of equal importance, the results from the silaffin and Erkol suggest a direction for controlling the morphology of titania precipitation, opening new areas of research and application.

The results of this project suggest several areas for further research:

1) *New methods for creating self-decontaminating materials, opened up by the ability to precipitate titania using functionalized surfaces:* Antimicrobial and reactive agents could be co-precipitated with the titania onto amine functionalized surfaces as a reactive coating.
2) *Morphology:* More research on peptide secondary structure, as well as reaction conditions, is necessary to gain a better understanding of the mechanism's underlying oxide formation and morphology control.
3) *Precipitation of alumina using biomimetic agents:* The results presented here suggest it is possible to precipitate alumina, but more research is needed to clearly understand this ability and its benefits.
4) *The biomimetic approach to forming metal oxides:* This is still a nascent field of research. The formation of other potentially useful oxides under benign reaction conditions has potential with this technique. These include zinc oxide for photonics applications and heterogeneous oxides, such as barium titanium oxide, for capacitors, displays, and other devices

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